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Ecdysteroid Treatment Delays Ecdysis in the Lobster, *Homarus americanus*

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Abstract. Premolt stage D_3 of juvenile lobsters, *Homarus americanus*, was further divided into five substages according to the degree of cuticle digestion along the dorsal midline of the carapace and on the dorsal surface of the merus of the chelipeds. The mean times to ecdysis for intact lobsters (5.5 ± 1.1 g) at each substage were 71.4, 57.7, 30.0, 16.1, and 6.6 h, respectively. The level of ecdysteroids dropped continuously during stage D_3, from 0.5 μg/ml at substage 1, to less than 0.1 μg/ml at substage 5. Injections of 20-hydroxyecdysone (20-HE) (1.0 or 5.0 μg/g) delayed ecdysis in animals receiving an injection at substages 3 or 4, but not 1, 2, or 5. The staging method can be applied to eyestalk-ablated (ESX) lobsters as well; but those animals complete stage D_3 and molt much more rapidly. In addition to the time of ecdysis, the rate of development (based on the degree of cuticle digestion) in both intact and ESX lobsters was decreased by injections of 20-HE. We conclude that decreased ecdysteroid titers in the hemolymph of lobsters is a prerequisite to the initiation of ecdysis, and that rates of development during stage D_3 are regulated negatively by ecdysteroids. We suggest that the time of ecdysis is controlled in lobsters through the regulation of the rate of decline of ecdysteroid titers.

Introduction

Like most other crustaceans, lobsters (*Homarus americanus*) increase their body size by molting. Before each molt, the inner layers of the old cuticle are reabsorbed, and the outer layers of the new cuticle are synthesized underneath the old one (Skinner, 1985, for review). Shedding the cuticle at ecdysis is accompanied by a sudden increase in body weight because of rapid water uptake (Mykles, 1980). This water occupies the space made available by the larger and more pliable new cuticle synthesized before molt. The enlarged space is needed for subsequent tissue growth prior to the next molt. This cyclic event, which is repeated throughout the life of many crustaceans, is under the control of ecdysteroids (molting hormones) (Chang, 1989, for review).

In lobsters, as in many other crustaceans, the ecdysteroid level rises during early premolt and reaches its peak at late stage D_2, or early D_3, then drops to a low level immediately before ecdysis (Chang and Bruce, 1980, 1981; Chang and O'Connor, 1988). A similar ecdysteroid profile has been found in insects (Steel and Vafopoulou, 1989, for review). In both insects and crustaceans, the rising titers of ecdysteroids initiate the cascade of events that culminate in ecdysis (Skinner, 1985; Chang, 1989). In addition, decreasing titers of ecdysteroids in late premolt are necessary for the initiation of ecdysial behavior in some insects (Sláma, 1980; Truman *et al.*, 1983; Reynolds, 1986; Zdarek and Denlinger, 1987).

Injections of ecdysteroids during late premolt inhibit ecdysis in some amphipods (Graf, 1972a, b). Similar results, though, have not been obtained in other crustacean species (Skinner, 1985, for review). One possible explanation for these negative results is that injections were not made at the right time. In an insect, *Manduca sexta*, injections made before or after the critical period have no effect on the time of ecdysis (Truman *et al.*, 1983). The lack of a precise staging method for crustaceans in late premolt may have prevented the discovery of an analogous critical period.

In insects, decreasing titers of ecdysteroids prior to molt not only regulate the time of ecdysis itself, but also modulate the rate of premolt development (Schwartz and Truman, 1983). Although crustaceans can regulate the

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time of ecdysis to varying degrees (Skinner, 1985), a study comparable to that in insects has not yet been conducted.

During the last few days before ecdysis, certain regions of the cuticle are digested to a greater extent than others, enabling the lobster to shed its old exoskeleton. In our study, we developed precise staging criteria for stage D_3 lobsters. The time of ecdysis during the last three days of the molt cycle could be predicted on the basis of these criteria. In addition, the effect of injecting 20-hydroxyecdysone on the rate of cuticle digestion and the time of ecdysis was investigated.

Materials and Methods

Maintenance of animals

Two families of full-sibling juvenile *Homarus americanus* (5.5 ± 1.1 and 3.2 ± 0.7 g wet weight; mean $\pm$ S.D.) were used for these experiments. The lobsters were raised in a semi-recirculating system at 20.0 ± 0.5°C on a diet of live adult brine shrimp (Chang and Conklin, 1983; Conklin and Chang, 1983). The photoperiod was 16L:8D.

Molt prediction

Before stage D_3, intact and eyestalk-ablated (ESX) lobsters (5.5 g) were staged based on the setal development of the pleopods (Aiken, 1973). Stage D_3 lobsters, about three days before molt, were further subdivided into five substages, based on the degree of cuticle breakdown along the dorsal midline of the carapace, and on the dorsal surface of the merus of the chelipeds (Table I). These are areas where extensive cuticle digestion occurs prior to ecdysis. For staging, the lobster's dorsal carapace was wiped dry and evaluated with a dissecting microscope.

Exogenous ecdysteroid injection

Intact lobsters (5.5 g) at various substages of stage D_3 received a single injection (0.2, 1.0, or 5.0 μg/g wet weight) of 20-hydroxyecdysone (20-HE; Rohto Pharmaceutical; purity checked by high-performance liquid chromatography before use). Control animals received vehicle only (lobster saline; Mykles, 1981).

ESX lobsters (3.2 g) were used for one injection study. Both eyestalks were removed from stage B–C animals, with fine scissors, about 5–10 days after molting. When animals were approaching the first postoperative molt, they were staged by the same method described above and injected with lobster saline, either containing 20-HE or not. Only one dose of 20-HE (1.0 μg/g wet wt.) was used per animal in these experiments. The time between injection and ecdysis was recorded on time-lapse video with a time stamp.

Endogenous ecdysteroid determination

Before injection, a hemolymph sample (25 μl) was taken from each animal and the content of its endogenous ecdysteroids determined quantitatively by radioimmunoassay (Chang and O'Connor, 1979); the IB-4 antiserum from Dr. W. E. Bollenbacher was used (University of North Carolina, Chapel Hill).

Rate of development

The effect of ecdysteroid titers on the rate of progression through the D_3 substages was investigated. Lobsters (5.5 g for both intact and ESX animals) received an injection of 20-HE (1.0 μg/g), once per day, starting from substage 1 of stage D_3. The rates of development were recorded twice daily based on criteria described in detail below.

Results

Stage D_3 was divided into five substages according to the degree of the cuticular digestion in the regions shown in Figure 1. At substage 1, only the posterior region of the dorsal midline of the carapace shows signs of digestion. This is manifested as a narrow crack that starts at the posterior end of the midline and proceeds anteriorly about one-third of its total length.

At substage 2, another narrow crack appears in the dorsal midline of the carapace. It starts from the anterior of the carapace and proceeds posteriorly to about one-third of the total length of the dorsal line.

At substage 3, the narrow crack forms along the entire dorsal midline. At substage 4, the crack widens to occupy the entire width of the dorsal midline along the posterior two-thirds of its length. At substage 5, the crack widens along the entire length of the midline. Also, the dorsal surface of the merus of each cheliped becomes soft (Table I).

The times to ecdysis for animals in substages 1 to 5 are given in Table I. Although the molt-staging technique can be applied to ESX lobsters, these animals spend much less time in each substage (Table I). The levels of the ecdysteroids in the hemolymph of intact lobsters drop continuously during stage D_3, from 0.55 μg/ml at substage 1, to less than 0.1 μg/ml at substage 5 (Fig. 2).

The role of ecdysteroids in regulating the time of ecdysis was examined by injecting stage D_3 lobsters with various doses of 20-HE. Lobsters were divided into the five substages as described. Each animal received a single injection of lobster saline, with or without 20-HE. A low dose (0.2 μg/g) of 20-HE did not significantly delay ecdysis in animals receiving the 20-HE injection at any substage (Fig. 3). High doses (1.0 and 5.0 μg/g) delayed ecdysis in animals receiving an injection at substages 3 and 4, but not 1, 2, or 5 of stage D_3.

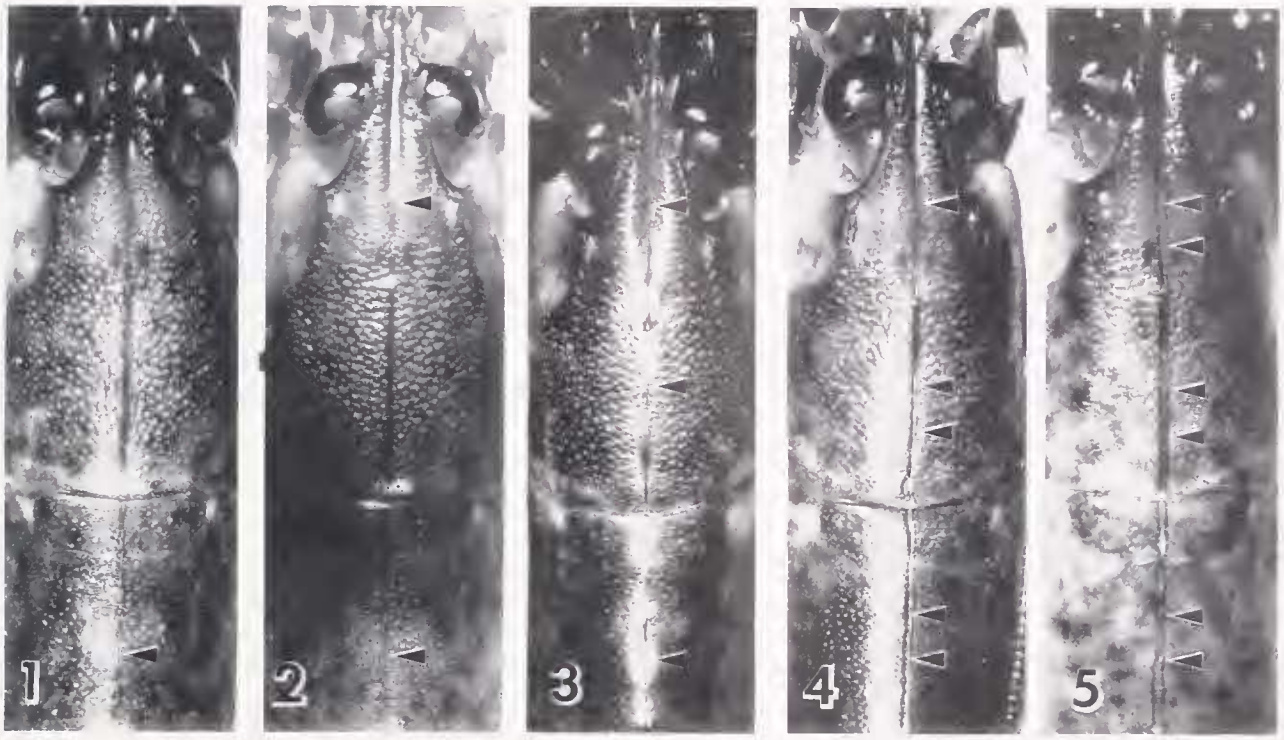

Figure 1. Carapace of juvenile lobsters, *Homarus americanus*, showing the progress of cuticular digestion along the dorsal midline. Single arrow indicates slight digestion. Double arrows indicate heavy digestion (see Table I). The numbers indicate the substages of D_3. Field of view is approximately 7 × 20 mm.

Ecdysis of ESX lobsters receiving an injection of 20-HE was also delayed (Fig. 4). Ecdysis was delayed in all lobsters receiving a 20-HE injection at substages 1 or 2, and in seven of nine lobsters at substage 3, but in none of the lobsters at substages 4 and 5. The unresponsiveness of the two lobsters (at substage 3) to the 20-HE injections might have been due to lower ecdysteroid titers (0.18 and 0.28 μg/ml) than the other seven animals (range of 0.35 to 0.66 μg/ml). These two animals may have been approaching substage 4.

Injection of 20-HE not only delayed ecdysis, but also depressed the rate of development. This was assayed by the degree of cuticle digestion over time in both intact and ESX lobsters (Fig. 5).

Table I

Substages of D_3 based upon cuticle digestion in both intact and eyestalk-ablated juvenile Homarus americanus *(5.5 g) at 20°C*

	Cuticle digestion at				Hours before ecdysis (n) (mean ± S.E.)	
	Dorsal midline of carapace					
Substage of D_3	Anterior region	Central region	Posterior region	Dorsal surface of merus of cheliped	Intact	Ablated
1	−	−	+	hard	71.4 ± 9.5 (9)	32.4 ± 8.2 (8)
2	+	−	+	hard	57.7 ± 4.0 (9)	21.8 ± 5.4 (6)
3	+	+	+	hard	30.0 ± 6.0 (6)	10.8 ± 5.0 (6)
4	+	++	++	hard	16.1 ± 2.0 (7)	6.0 ± 1.0 (4)
5	++	++	++	soft	6.6 ± 0.7 (7)	4.2 ± 2.3 (5)

− Indicates digestion absent, + indicates slight digestion (width of the crack is less than the width of the dorsal line), ++ indicates heavy digestion (width of the crack is wider than the width of the dorsal line).

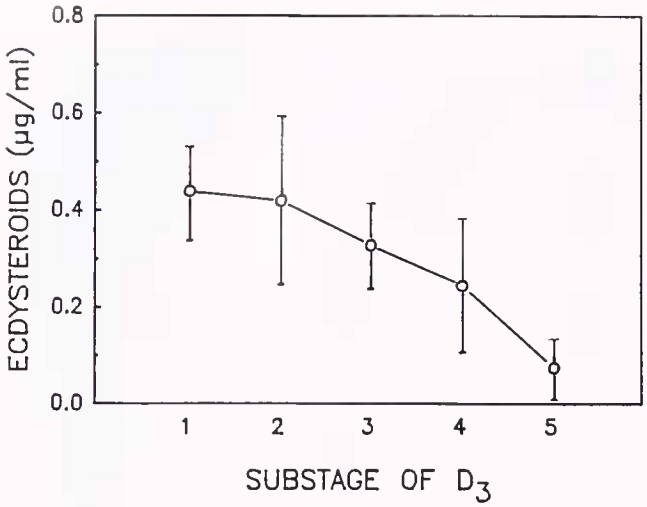

Figure 2. Ecdysteroid titers in the hemolymph of intact 5.5 g lobsters, plotted against substage of D_3 (means ± 1 S.D.). Each datum point represents 10–19 animals.

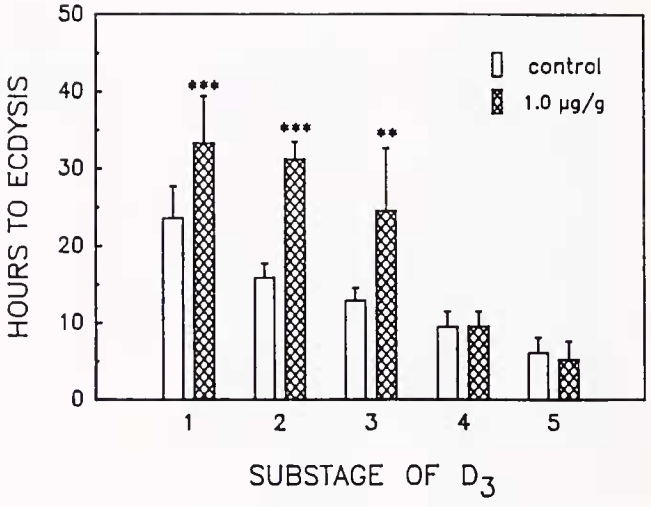

Figure 4. The effect of 20-HE injection (1.0 µg/g) on time of ecdysis in eyestalk-ablated 3.2 g lobsters (means ± 1 S.D.). Animals received a single injection of saline with or without 20-HE at the stage indicated. The time of ecdysis was recorded using time-lapse video. Asterisks (** and ***) indicate significant differences ($P < 0.01$ and 0.001, respectively). Each group contained 6–12 animals.

Discussion

Intensive cuticle digestion along the dorsal midline of the carapace starts about three days before ecdysis and permits lateral expansion of the carapace at ecdysis. The intensive cuticle digestion on the dorsal surface of the merus of the chelipeds permits lobsters to withdraw their large chelipeds through the narrow basiischial joints. Cheliped withdrawal is also facilitated by the differential degeneration of cheliped muscle tissue (Mykles and Skinner, 1981) and active water uptake (Mykles, 1980; Cheng, 1990).

Our molt-staging technique, based on cuticle digestion, reliably predicted the time of ecdysis, which was critical for this study. This technique should also be applicable to other species, for research and for the commercial production of soft-shell crustaceans.

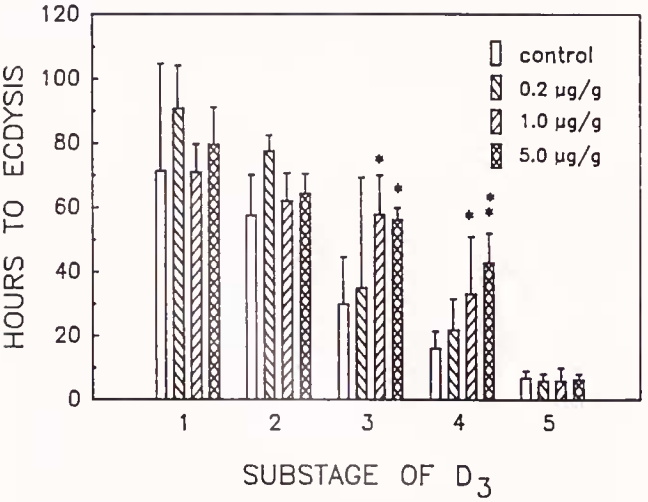

Figure 3. The effect of injection of various concentrations of 20-HE (0, 0.2, 1.0, or 5.0 µg/g) on time of ecdysis in intact 5.5 g lobsters (means ± 1 S.D.). Animals received a single injection of saline with or without 20-HE at the stage indicated. The time of ecdysis was recorded using time-lapse video. Asterisks (* and **) indicate significant differences ($P < 0.05$ and 0.01, respectively). Each group contained 7–9 animals.

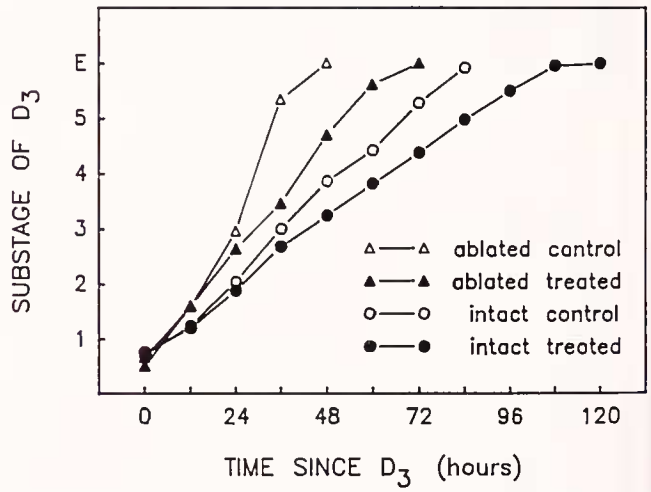

Figure 5. The effect of 20-HE injection on the rate of cuticle digestion in intact and eyestalk-ablated 5.5 g lobsters. Animals received an injection of saline with (treated) or without (control) 20-HE (1.0 µg/g) every 24 h, starting from substage 1. The stage of cuticle digestion was subsequently checked every 12 h. Each group contained 7–12 animals. E indicates ecdysis.

We observed that ecdysteroid titers continued to decline during the last three days of the molt cycle. Similar results have been reported for juvenile lobsters in our laboratory (Chang and Bruce, 1980, 1981). The mechanisms responsible for the decline are not clear, but a decrease in the rate of production of ecdysteroid by the Y-organ may be the predominant means of controlling the declining hormone titers. Evidence for this is the observation that the secretory rate of ecdysteroids by the Y-organs of *Pachygrapsus crassipes in vitro* was correlated with circulating ecdysteroid concentrations (Chang and O'Connor, 1978). Both negative and positive short-loop feedback mechanisms have been demonstrated in the prothoracic glands of *Manduca sexta* (Sakurai and Williams, 1989), and similar mechanisms may operate in Y-organs of crustaceans as well. In addition, increased rates of degradation of ecdysteroids in late premolt may play an important role in regulating the declining titers of ecdysteroids (Snyder and Chang, 1991).

High doses of 20-HE (1.0 or 5.0 μg/g) delayed molt in intact lobsters receiving injections at substages 3 or 4. This observation is consistent with the observation in insects that the decline in circulating ecdysteroids must precede the occurrence of ecdysis (Sláma, 1980; Truman *et al.*, 1983; Reynolds, 1986; Žďárek and Denlinger, 1987). In addition, the delay of ecdysis by exogenous ecdysteroid in lobsters is similar to observations in insects, where there is a critical period when animals are most sensitive to hormonal treatment (Truman *et al.*, 1983; Žďárek and Denlinger, 1987).

Intact lobsters become insensitive to exogenous 20-HE when they reach substage 5, as development nears completion (within 7 h before ecdysis), and as the circulating level of the hormone declines to its low level (less than 0.1 μg/ml). This insensitivity suggests that the ecdysial programs have already been triggered. In *M. sexta*, this trigger includes the acquisition of the sensitivity to and release of eclosion hormone (EH), which coordinate ecdysial behavior and related physiological events (Truman, 1985). Although Truman *et al.* (1981) found EH activity in insects from five non-lepidopteran orders, and quantified it with a *Manduca* bioassay, direct demonstration of the existence of an EH-like factor capable of initiating ecdysis has not been reported in any non-lepidopteran.

In crustaceans, an exuviation factor analogous to EH has been proposed, based on the observation that ecdysteroid injection blocked ecdysis in some amphipods (Graf, 1972a, b; Charmantier and Trilles, 1976). There is no direct evidence, however, for EH in crustaceans. Peptide extracts from brains and thoracic ganglions of pre- and postmolt crabs were assayed for EH activity, all with negative results (Cameron, 1989). Crustaceans and non-lepidopteran insects may not use an EH-like factor to initiate ecdysis, but instead directly rely on the declining titers of ecdysteroids to trigger ecdysial behavior.

The declining ecdysteroid titers at late premolt influence other aspects of development, in addition to the time of ecdysis. As shown in Figure 5, cuticular digestion was delayed by multiple injections of 20-HE. These results suggest that the events in late premolt are negatively modulated by ecdysteroids. This is consistent with observations in insects (Schwartz and Truman, 1983). In *M. sexta*, however, morphological development was completely suspended by continuous ecdysteroid infusion. The negative effect of ecdysteroids on late premolt events is in contrast to their positive effect on development at other molt stages in both insects and crustaceans.

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